Symbiosis

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Mijnheer de rector, dames en heren, ladies and gentlemen, dāmas un kungi, hartelijk welkom bij mijn oratie getiteld "*Symbiosis"*. Zoals jullie zien, is de titel in het Engels. Ik zal de oratie vanwege de buitenlandse vrienden en familieleden in het Engels voortzetten. Ik heb geprobeerd om de verschillende processen zo eenvoudig mogelijk uit te leggen en visueel weer te geven, in de hoop dat mijn verhaal ook voor leken tussen jullie goed te volgen is.

First of all, I would like to explain the title and the reason for choosing this title for my lecture. "Symbiosis" (from Greek "living together") is defined as a close and often a long-term relationship between two species. Originally only mutualistic relationships were considered symbiotic, while the current consensus among biologists is to regard all forms of relationships as symbiotic. These include mutualism, commensalism and parasitism. In parasitism one species benefits while the other is harmed, such as human head lice, living on the scalp and feeding from the blood of the host who suffers from itching. Commensalism (from Latin, *com-* and *mensa*, meaning "sharing a table") involves two species of which one benefits from the other, while the other has neither beneficial nor harmful effects from this relationship. Such a relationship occurs between a remora fish and a shark: the remora fish sucks itself to the surface of the shark with a suction cup-like organ and lifts along with the shark in search for a prey, feeding on the remains of the prey. In mutualistic relationships both individuals benefit from the relationship. For instance, when a Nile crocodile has food remnants stuck between its teeth, it lies with its mouth open and waits for a plover bird to land on its tongue and to feed on the remnants, at the same time cleaning the crocodile's teeth.

In this lecture, I will first address the role of symbiosis in the evolution of life on Earth. Then I will move to humans and their microbial symbionts, starting with the microbiome of the gut. Thereafter I will focus on my research objects – the symbionts of human oral cavity or oral microbiome. Here I will introduce you to

the topic of oral microbial ecology and to the role our microbial symbionts have on our wellbeing. Finally I will highlight my personal symbiosis with research and ACTA for nearly 20 years.

The role of symbiosis in the evolution of life on Earth

"Life did not take over the globe by combat, but by networking" (Margulis & Sagan, 1986) are the words of prof. dr. Lynn Margulis (1938-2011), a controversial visionary in biology. She was an evolutionary biologist who pioneered the concepts of endosymbiosis and symbiogenesis. To understand her theory, let's move back in time for ~2.9-4 billion years, the estimated origins of life on Earth. Around this time, under the reducing conditions of the primitive terrestrial atmosphere, the first prokaryotic cells – anaerobic bacteria – arose. For billions of years they were the only inhabitants of our planet. At some point, the first photosynthetic microorganisms appeared. They were able to convert light energy into chemical energy and oxygen. When oxygen became relatively abundant in the atmosphere (~1.9 billion years ago), some bacteria adapted to respiring oxygen and generating energy directly from it - they became aerobes. In her endosymbiosis theory, prof. Margulis proposed that the first step in the origin of eukaryotes was related to a symbiotic relationship between an anaerobic amoeba-like microorganism and an aerobic bacterium.

The amoeba-like microorganism ingested the aerobic bacterium without digesting it. The intracellular presence of the bacterium provided a survival advantage to the host: it produced energy from oxygen. This endosymbiosis led to the evolution of mitochondria – the energy factories of any eukaryotic cell. Similarly, assimilation of photosynthetic bacteria by endosymbiosis has led to the evolution of chloroplasts, now present in plants and algae. Chloroplasts produce oxygen that all animals, including humans, breathe.

The long history of shared ancestry and alliances between humans and microbes is reflected in their genomes. Analysis of the large number of full genome sequences presently available reveals that most life forms share approximately one third of their genes, including those encoding central metabolic pathways (McFall-Ngai et al, 2013). Many human genes are homologs of bacterial genes, mostly derived by descent, but occasionally by gene transfer from bacteria.

The symbiosis between microbes and their human host

Let's keep in mind that besides their common ancestry with microbes, humans have evolved in a continuous presence of and in symbiosis with microbes. This brings me to the next part of my lecture: *the symbiosis between microbes and their human host*. Our body hosts approximately as many microbial

cells as human cells. The microbial cells on and in our body carry genes that outnumber our own genes by a factor of 150.

For numerous chemical reactions (e.g., nutrient breakdown) we lack our own tools - the enzymes that are specialized in these reactions - and we have to rely on our symbionts. As much as a third of our metabolome – i.e., the diversity of molecules carried in our blood – has a microbial origin (McFall-Ngai et al, 2013). In return, microbes receive their favourite food and living conditions from us. Due to the mutualistic symbiosis there is a continuous host-microbiome crosstalk. Our microbiome forms the first line of defence and prevents establishment of exogenous microorganisms. It trains our immune system to recognize a 'friend' from a 'foe': it down-regulates pro-inflammatory response towards commensals and stimulates the response against invaders.

But how do we get our microbes? Microbiome is acquired during or right after birth through vertical transmission from mother and through horizontal transmission from siblings and other people in the same environment. It is affected by the mode of birth and the method of feeding during early stages of life. Acquiring and maintaining the healthy microbiome is of high importance, since dysbiosis of the gut microbiota is associated with the pathogenesis of both intestinal (e.g. inflammatory bowel disease, irritable bowel syndrome and coeliac disease) and extra-intestinal disorders (e.g. allergy, asthma, metabolic syndrome, cardiovascular disease and obesity).

The critical role of microbiome on normal functioning of the host has been evidenced by studies with germ-free animals raised in a sterile environment. Mice that are raised germ-free have an altered immune system, heart, lungs, lymph nodes, metabolism, reproductive ability and show even altered brain development and behaviour.

Not only the mere presence but also the composition of microbiome has been shown to affect the host. When gut microbiota from an obese human were put into a germ-free mouse with no changes to the mouse's diet, the metabolism of the mouse changed and mouse gained weight (Ridaura et al., 2013). If the microbial sample was from a lean human, the weight gain did not occur. Interestingly, once the mice already harboured the microbiota from a lean human, the microbiota from the obese individual could not colonise the gut of these mice and the mice remained lean.

The study on mice microbiome reported by Bercik and colleagues (2011) has become a classic example on communication between the gut and the brain – a so called "gut-brain axis". They used a behavioural test that measures how long it takes until an animal jumps off an elevated platform. The experimental animals

were two types of mice – one very relaxed and exploratory, the other – very nervous and frightful. Then they gave to the nervous mouse a faecal microbiome of the relaxed mouse. This resulted in changed behaviour of this mouse: it became more relaxed and exploratory. Vice versa – the exploratory mouse, after receiving the microbiome of the nervous mouse, became more stressed and nervous. How the communication between the gut and the brain occurs is not clear yet, but it is likely to involve neural, humoral and inflammatory pathways.

Our oral symbionts

After hearing about these quite impressive examples from the gut microbiome research you might wonder, why we should pay attention to the microbiome of the oral cavity, which is just a small part of the gastrointestinal system. Now we have approached the main part of my lecture where I will try to persuade you that *our oral symbionts are all but insignificant*.

The oral microbiome consists of hundreds of different microbial species, including bacteria, fungi, Archaea, viruses and protozoa. Once established, oral microbiome remains incredibly stable. Daily perturbations such as mechanical removal by chewing and toothbrushing, exposure to antimicrobial agents in oral care products and foods, temperature gradients, host factors such as saliva and immune system, do not seem to disturb the balance of the oral ecosystem. Even a single exposure to a course of antibiotics did not affect the salivary microbiome of healthy volunteers, while the faecal microbial communities did not recover until several months since the antibiotics (Zaura et al., 2015). This stability evidences the long-term symbiotic relationship of our oral microbes with their habitat – the oral cavity – and their pivotal role in our wellbeing.

The oral cavity, lined by mucosa, is an important part of the gastrointestinal tract. The same functions mentioned in respect to gut, apply also to oral microbes. The oral cavity is exposed to the outside environment and potential intruders. Therefore "the gatekeeper" function of the oral microbiome is very important. Interestingly, in new-borns, the oral microbiome seeds the gut microbiome: at first the gut microbiome resembles that of the oral cavity. Only in about two weeks time it diverges towards a gut-specific community (Costello et al., 2013).

Besides seeding the gut microbiome in new-borns, oral microbes make other unique contributions in their symbiotic relationship with us. The most remarkable example is their contribution to the nitric oxide homeostasis in our body (Hezel & Weitzberg, 2015). Nitric oxide is a signalling molecule that is involved in many regulatory processes of the body. One of its functions is vasodilatation or relaxation of blood vessels, which is important for the

regulation of blood pressure. Nitric oxide can be obtained in series of chemical reactions from dietary nitrate. Foods as spinach and red beets are rich with nitrate. However, there is one problem: our body cells do not possess genes that encode for the enzyme necessary to reduce nitrate to nitrite. Instead, we kindly borrow this enzyme from the toolbox of our oral symbionts – oral bacteria. One would argue that the food is chewed and swallowed before oral microbes get any chance to get their enzymes ready and to do the work for us. Well, the evolution of the host has invested in an elegant solution for this. Dietary nitrate that is swallowed, passes the stomach and enters the intestines. There it is absorbed in blood and reabsorbed, concentrated and excreted by salivary glands in saliva. While we are sleeping, the oral bacteria can complete their job after all.

As a result of thousands of years of mutualistic symbiosis, at health, the human microbiome is in balance with its host and the symbiosis is mutualistic. I would like you to look at the oral cavity as an ecosystem. An ecosystem is a community of living organisms in conjunction with the nonliving components of their environment (like air, water and mineral soil), interacting as a system. An example of a complex ecosystem is a tropical rainforest. If the trees are cut, the entire ecosystem changes. The rainforest loses its floral and faunal species richness and becomes vulnerable to environmental challenges such as heavy rainfalls. Just think of avalanches of mud, taking entire villages down with them.

The oral ecosystem responds similarly to an ecological stress. At health, it is stable and in balance with the host. If an ecological pressure, such as frequent exposure to sugars, lasts long enough, the ecosystem loses its balance towards acid tolerant and acid producing microorganisms. Their symbiosis with the host changes its mutualistic character and becomes antagonistic to the host. With time this might lead to dental decay. On the other side of the spectrum of ecological disturbances is the inflammatory response of the host to microbes. This is often related to poor oral hygiene, but also to an aggravated host response to its own microbiome. As a result, proteolytic and anaerobic microbial taxa are selected that promote additional inflammatory response of the host and that may lead to a periodontal breakdown.

Decades have been spent in research on the role of microbes in the aetiology and pathogenesis of oral diseases – the antagonistic spectrum of the host-microbe symbiosis. Only recently the necessity of understanding the mutualistic symbiosis at health has received attention.

In a large academia-industry collaborative project within the Oral Health theme of the 'Top Institute of Food and Nutrition" we have tried to unravel the interrelationships between microbial factors and saliva – the environment provided by the host. For this we performed a study on a group of 268 orally and

systemically healthy young adults. We obtained clinical and behavioural information such as their dietary preferences, and collected biological samples such as saliva, which was assessed for microbial and metabolic composition and biochemical properties. When we analysed the salivary microbial composition of these healthy individuals, they appeared to split into five distinct groups, each corresponding to a different type of microbial community. Some were more rich in bacteria called streptococci, some – in prevotellas and veillonellas. Some communities harboured fewer and some – more bacterial species than the rest. Then we combined the microbial composition results with the rest of the data on salivary metabolites and salivary biochemistry. This resulted in the following findings. The microbial communities in these healthy oral cavities correlated with one of two metabolic directions - they were either saccharolytic, meaning that they preferred metabolizing sugars, or they were proteolytic, meaning that they would rather use proteins as their energy source. Additionally, based on biochemical properties such as pH and activity of specific salivary enzymes, some of the microbial community types or ecotypes appeared to be more specialized than others to perform certain metabolic functions (Zaura et al., 2017). For instance, the samples with bacteria specialized in sugar metabolism also showed the lowest microbial diversity and low salivary pH. In contrast, the group presented with highly complex microbial communities specialized in protein breakdown, had high content of albumin in saliva and high salivary pH.

The presence of the ecotypes suggests that "a one size fits all" approach in prevention and oral care will not work. For example, there are oral care products that contain substances that promote acid neutralization and increase oral pH. Arginine is one of such substances. It is an amino acid that is metabolized by oral bacteria into ammonium and results in a pH increase. This in turn allows the oral ecosystem to recover from acid stress. However, based on the metabolic preference by different oral ecotypes, pH increase will be only beneficial for the saccharolytic types. In contrast, enhancement of the pH rise in the proteolytic ecotypes will only promote further specialization towards a more proteolytic community - an effect that might not be desirable at all.

Secondly, the ecotypes that showed the highest specialization might be more susceptible towards disease: the proteolytic ecotype – towards gingivitis and periodontitis, the saccharolytic one – towards dental caries. By performing longitudinal studies – thus by following individuals of various ecotypes in time – we will be able to determine if the differences that we observed are early warnings for a disturbed ecosystem and are valid preclinical signs of a disease.

Let's move back to examples from nature again. In October 2013 there was a heavy storm in the Netherlands. About 30 trees along one road did not survive the storm while several other trees along the same road remained undamaged.

Did they have deeper roots, lower height, less wide branches allowing them to withstand the wind? Most likely all of above contributed to their resilience to stress.

During our life, the oral ecosystem undergoes various changes that are influenced by the stage of life we are in, for instance, being an infant and getting the first teeth, or entering puberty with hormonal fluctuations, or reaching an old age and experiencing decreased salivary flow and function. At each of these stages the oral ecosystem needs to adapt to the changing environment and to stresses it is exposed to. The individual variables that contribute to this ecosystem, such as dental hard tissue, saliva, microbiome, immune system, mucosal tissues and the endocrine system, all contribute to the resilience of the ecosystem facing stress (Zaura and Ten Cate, 2015). To date, the only variable that we could claim as being well studied is the dental hard tissue or tooth enamel. We know what we should do to enhance its resilience against acid stress: we have to expose the enamel to fluoride. We know at which salivary pH and calcium levels the enamel will dissolve or repair. In contrast, we have no idea when, for instance, a microbial community will change its structure and composition and how to restore the balance after these changes have occurred.

To understand these processes, we have to follow the oral ecosystem of healthy individuals in time, preferably starting at a young age, and to measure the various variables of the ecosystem. These are very laborious studies, which require large funding and collaborative networks of experts. In such collaboration with prof. dr. Margherita Fontana at the University of Michigan and National Institutes of Health, we are going to assess the oral ecosystem of children that are growing up in socio-economically deprived families in US. These are children known to be at high risk for dental caries. They are followed in time until they reach 4 years of age. Their dental status, saliva and microbial composition of dental plaque are assessed together with their dietary and oral hygiene habits. In the vast majority of these children the ecosystem collapses and this results in dental decay, while some children remain healthy. By combining the clinical, behavioural and biological variables collected in time we hope to be able to determine what makes some children more resilient towards disease than others and how we can predict the onset of the disease.

Nobody likes experiencing pain and discomfort or even loosing teeth due to caries or periodontal disease – reasons important enough for motivating an individual to take good care of his or her oral health. Yet another reason why a healthy oral ecosystem is of great importance is that it is interconnected with the rest of the body.

The online lecture – a kind of a Ted-talk in Dutch – by prof. dr. Erik Scherder from Vrije Universiteit Amsterdam was announced in a Dutch newspaper with the following headline: "Why is vanilla pudding destroying our brain?" In his lecture he explains how chewing, just like physical exercise, can slow down aging-associated deterioration of memory. The importance of a good-functioning dentition was recently shown in the outcome of this systematic review: Poor mastication was associated with lower cognitive function and increased incidence of dementia (Tada & Miura, 2017).

The saying: "Never look a gift horse in the mouth" we can find back both in Dutch: "Je moet een gegeven paard niet in de bek kijken", as well as in Latvian: "Dāvinātam zirgam zobos neskaties". The meaning of this proverb is the same in all three languages: A gift should not be judged but accepted as it is. Interestingly though, all three languages use the oral status of a horse for this saying. This is due to a common understanding that judging the oral health of a horse reveals the overall health status of the animal.

Another example on how general health has been linked to the physical appearance of oral tissue comes from China. Judgment of the appearance of a tongue and tongue coating has been an important part of tongue diagnosis for thousands of years in traditional Chinese medicine.

Currently there is enough scientific evidence to say that poor periodontal health is associated with increased risk for atherosclerotic cardiovascular disease, adverse pregnancy outcomes and type-2 diabetes. Some association has been found also between periodontitis and metabolic syndrome, obesity, COPD, pneumonia, chronic kidney disease, rheumatoid arthritis, cognitive impairment and cancer. The involved mechanisms are thought to be by chronic inflammatory response as well as by bacterial spread in the body. For instance, periodontal bacteria have been found in placenta and amniotic fluid of low birth weight, premature and stillbirth pregnancies. Oral bacteria have been located at other distant sites from the oral cavity such as colorectal carcinoma, pancreatic cancer and atherosclerotic plaques.

Thus far, when oral microbes are found elsewhere in the body than in the oral cavity, they have lost their mutualistic symbiotic relationship and enter an antagonistic symbiosis with the host. Therefore I was immensely intrigued by the presentation by dr. Kjersti Aagaard from Baylor College of Medicine, US, during the Human Microbiome Project congress in China. She reported on her study of placenta microbiome from over 300 healthy pregnancies. It appeared that placentas of healthy pregnancies were not sterile as generally assumed but harboured their own microbiome (Aagaard et al., 2014). When she compared the microbes found in placenta with the microbes from different body sites in

healthy humans, she concluded that the placental microbiome was most similar to the microbiome from tongue and tonsils. When asked for the fate of placental microbes, she suggested their potential role in seeding the foetus with microbes already before birth.

Back at ACTA I brainstormed with my colleagues Elena Nicu, Bart Keijser and Bastiaan Krom and we came up with our own hypothesis on why the oral microbes would travel all the way to the placenta (Zaura et al., 2014). During pregnancy, due to hormonal effects, gingiva becomes swollen and bleeds easily. This would allow oral bacteria to enter the blood stream, either by themselves or by lifting with dendritic cells. They would follow the blood stream towards the most actively growing organ in the body at this time – the placenta and get trapped inside placental tissue. On the foetal side, peripheral lymphoid tissues are being developed. Naïve T cells travel to the placenta and acquire antigen information from the bacteria or their fragments. This way they would become educated T cells or T regulatory cells once they return to the foetus.

At birth, the immune cells would be well educated on which microbes are friends and which – enemies. These cells would help selecting the right microbes for the settlement – the microbes that we have evolved with in thousands of years in a mutual symbiosis and not a random set of trespassers that just happened to be in the neighbourhood around birth. If our hypothesis holds true, and there is this crosstalk between the microbiome of the mother and the foetus, a healthy oral ecosystem of a mother would become extremely important in shaping the healthy development of the immune system and consequently – good general health of her offspring. To test this hypothesis we will need to set up interdisciplinary collaborations from the fields of microbiology, immunology, gastroenterology, gynaecology and neonatology.

In summary, we live on a bacterial planet, in a symbiosis with microbes. This symbiosis is mutualistic at health, but becomes antagonistic once the balance is disturbed. I hope that with this lecture I have convinced you about the importance of the healthy oral ecosystem. We know all about diseases, but we know hardly anything about what keeps someone healthy. In our future research we should focus on understanding the processes that steer the acquisition of a healthy microbiome and sustain the maintenance of oral health.

My personal symbioses

When I originally thought about this lecture, I aimed at spending most of the time on acknowledgments. The more I got into the preparation for this talk, the less time remained for thanking everyone I should.

Ten eerste, dank ik het college van bestuur van de Vrije Universiteit, de leden van de benoemingscommissie en de decaan van het Academisch Centrum Tandheelkunde Amsterdam voor het in mij gestelde vertrouwen. Nooit eerder heeft de faculteit Tandheelkunde de positie van University Research Chair vervuld. Door mijn benoeming als URC heeft de Vrije Universiteit het belang van het onderzoek in tandheelkunde erkent. Hiermee is dit een welverdiende erkenning voor het werk van een ieder bij ACTA.

Mijn eigen onderzoek is verre van eenmanswerk. Ik sta hier dankzij het mentorschap, toegewijde steun en inbreng van velen. Ik zal hier een aantal mensen in bijzonder noemen, in de chronologische volgorde van hun intrede in de symbiose met mij.

Toen ik nog een middelbare scholier was, terug in de jaren tachtig van de vorige eeuw, ontmoette ik mijn eerste mentor in onderzoek – dr. Janis Vetra, later professor en rector magnificus van Riga Stradins University. Ik volgde het speciale biomechanica programma van het staatsgymnasium in Riga – iets wat op een technasium leek. Voor mijn onderzoeksopdracht hielp ik Janis Vetra – toenmalig chirurg-traumatoloog – bij zijn promotieonderzoek over een *Staphylococcus aureus*-werende coating voor heupprotheses. Pas ruim een decennium later besefte ik dat ik toen al met biofilmonderzoek bezig was geweest.

Toen ik al tandheelkunde studeerde in Riga, deed ik mee aan een selectie voor studeren in Zweden, aan het Karolinska Instituut. Hier ontmoette ik prof. Birgit Angmar-Månsson, nu emeritus professor van het Karolinska Instituut. Zij leidde toen de selectiecommissie en, nadat ik tussen de vijf gelukkigen belandde, was ze het hoofd van ons opleidingsprogramma in Stockholm. Dear Birgit, thank you for seeing more in me than I saw myself at that time. I wish you were able to come and be here today. I know how proud you are of me now, just as 15 years ago when I defended my PhD thesis and you were my opponent.

Tijdens mijn studie in Stockholm heeft mijn docent cariologie Joan Bevenius een enorme indruk op me gemaakt door wat ze tegen ons zei: “Even monkeys can be taught how to drill, you have to understand why!” – “Zelfs de apen kunnen leren boren, jullie moeten begrijpen waarom!” Dit ”waarom” heeft me sindsdien niet losgelaten.

Mijn volgende mentor – Marie Wahren, nu professor in experimentele reumatologie aan het Karolinska Instituut, ontmoette ik als student-assistent tijdens mijn studie tandheelkunde in Zweden. Ik mocht Marie helpen bij haar promotieonderzoek over het Sjögren syndroom in het celbiologie lab. Hier begon het knagen bij mij – ik zag dat er mensen waren die gepassioneerd “voltijds

onderzoekers" waren, terwijl ik over een tijd een best saai "voltijds clinicus" zou worden.

Maar mijn meest inspirerende mentor en persoon aan wie ik mijn 20 jarige symbiose met ACTA te danken heb, is Bob ten Cate, nu emeritus professor bij ACTA en Academie Professor van de KNAW. Bob en ik ontmoetten elkaar in de zomer van 1996 in Riga. Hij was een van de sprekers tijdens de ORCA zomerschool en ik – één van de vertaalsters van zijn lezingen, naast mijn werk als tandarts. Bob kende Birgit Angmar-Månsson, en wist over het project met de Baltische studenten. Hij begon meteen over mijn toekomstplannen. Ik zei dat ik onderzoek wilde doen, maar dat er geen geld voor was. Toen waren het nog de "gouden tijden" in Nederland en hij zei meteen: "Kom naar Amsterdam, wij hebben geld!", en stelde verschillende onderzoeksprojecten voor. De rest is geschiedenis. Lieve Bob, je haalde me naar ACTA als jonge clinicus, volledig onervaren in het onderzoek dat bij Experimentele Preventieve Tandheelkunde werd gedaan. Ik heb onmeetbaar veel van je geleerd, op heel veel aspecten van onderzoek en leven, en mijn dank aan jou is dan ook onmeetbaar.

Na mijn promotieonderzoek moest ik een eigen onderzoeks-niche vinden. Ik wou de complexiteit van de mond naar het lab brengen door speeksel als inoculum te gebruiken voor in vitro biofilms. Destijds was er maar één iemand in orale microbiologie die dat deed – dr. Chris Sissons van Otago Universiteit in Nieuw Zeeland. Dear Chris, thank you for sharing your passion in oral ecology. You gave me inspiration to go into this direction. You were the true pioneer in oral microbial ecology. Now not only our lab, but also the rest of the world uses the microcosm approach as a mainstream method.

Ergens rond 2006 begon mijn symbiose met Bart Keijser uit TNO, nu bijzondere hoogleraar bij ACTA in Orale Systeembiologie. Door jouw contact met Wim en onze samenwerking met TNO werden wij als groep meegenomen in de snel-evoluerende wereld van moleculaire detectietechnieken voor orale micro-organismen. Onze samenwerking heeft geresulteerd in hoog geciteerde artikelen en voor wereldwijde erkenning van ons werk.

In de prille kennismakingsperiode met de sequencing methode heb ik Sue Huse, toen nog bioinformaticus bij het Marine Biology Lab in Woodshole, VS, leren kennen. Dear Sue, you opened to me the world of bioinformatics and taught me how to modify your scripts and communicate with the computer cluster at SARA. Thank you for never saying no and always being there when yet another error message appeared on my screen and I got stuck with the scripts at this side of the ocean.

Ik wil even stilstaan bij een persoon die ons veel te vroeg heeft verlaten, dr Wilfred Röling. Beste Wilfred, jij hebt mij de beginselen van ecologie en microbiële profielanalyses geleerd. Je stille, altijd bedachtzame betrokkenheid heeft voor een symbiose gezorgd tussen de ecologen van de FALW, die in de aarde wroeten, en ons, die wroeten in monden. We moeten nog steeds de weg zonder jou leren vinden.

Het onderzoek dat ik doe kan alleen voortbestaan in een symbiose met mensen in het lab, in de kliniek, achter de computers en vergadertafels. Het prachtige van de afdeling preventieve tandheelkunde is dat we samen meer dan de som der delen zijn. Ik kan niet iedereen apart benoemen, maar zal voor een aantal toch een uitzondering maken. Ten eerste, Rob Exterkate: beste Rob, jij hebt je vanaf mijn eerste uur in Nederland over mij ontfermd, ook toen ik een moeilijke periode in mijn leven had. Dank je wel voor je steun! Monique van der Veen: beste Monique, wij delen nu al jaren een kamer. Jij bent altijd recht door zee en op jouw objectieve oordeel en advies kan ik altijd rekenen. Mark Buijs: beste Mark, al vanaf mijn promotietijd ben jij de sleutelfiguur geweest in het opzetten van onderzoeksmethodes cruciaal voor mijn werk – van setup van micro-electroden tot in Labview geprogrammeerde mond-modellen en tot het uitzoeken van alles wat met "amplicon sequencing" te maken heeft. Jij hebt inmiddels genoeg onderzoekservaring om zelf te promoveren. Ik zal je dan ook hiermee blijven plagen totdat het zover is. Bernd Brand: beste Bernd, dankzij jou hoef ik al jaren niet meer mijn hoofd te breken over scripts, terminals en clusters. Door het intensieve samenwerken aan onze mBio en ISME papers zijn we een goed geoliede tandem geworden. En natuurlijk onze groepsleider Wim Crielaard: beste Wim, ik zeg vaak aan mensen die vragen wat ik doe: "ik heb een hobby en krijg ervoor betaald." Jij geeft leiding op een subtiele manier en altijd met het oog op de ontplooiing van je medewerkers. Dank je wel voor de vrijheid om te groeien en de luxe om blijven mogen doen wat ik zelf leuk vind. Verder wil ik mijn eerste eigen AIOs bedanken – beste Chi, Eef, Jessica en Mercedes – niet alleen jullie maar ook ik heb met jullie enorm veel geleerd.

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